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application is a divisional of U.S. Application Serial No. 08/633,248 filed on April 18, 1996 (pending), which is a continuation-in-part of U.S. Application Serial No. 08/423,582 filed on April 18, 1995, which issued as U.S. Patent No. 5,795,725, each of which is hereby incorporated by reference, and from each of which priority is claimed --.

## IN THE CLAIMS

Please cancel claims 2-54 without prejudice to future prosecution. Please add the following claims.

55. An assay for determining the presence or amount of complexes of a cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds said cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C, and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said complexes, wherein said signal is at least a factor of two larger than a minimum signal resulting from said antibody binding to an equal number of free troponin components and/or troponin complexes which do not comprise said cardiac specific isoform of troponin, and wherein said signal is related to the presence or amount of said complexes in said sample.

56. An assay according to claim 55, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

57. An assay according to claim 55, wherein said signal is at least a factor of five greater than said minimum signal.

58. An assay according to claim 55, wherein the immunoassay comprises a sandwich immunoassay.

59. An assay according to claim 55, wherein said antibody is conjugated to a signal development element.

60. An assay according to claim 55, wherein said immunoassay comprises providing an antibody immobilized on a solid phase, the immobilized antibody binding said cardiac specific isoform of troponin.

61. An assay according to claim 55, wherein said antibody is conjugated to a signal development element, and wherein said immunoassay comprises providing an antibody immobilized on a solid phase, the immobilized antibody binding said cardiac specific isoform of troponin.

62. An assay according to claim 55, wherein said immunoassay provides a quantitative assay for said cardiac specific isoform of troponin.

63. An assay according to claim 55, wherein said cardiac specific isoform of troponin is cardiac specific troponin I.

64. An assay according to claim 55, wherein said cardiac specific isoform of troponin is cardiac specific troponin T.

65. An assay according to claim 55, wherein said assay provides a method for diagnosing a myocardial infarction.

66. An assay according to claim 55, wherein said cardiac specific isoform of troponin is cardiac specific troponin I, and said antibody specifically binds complexes comprising an oxidized form of cardiac specific troponin I.

67. An assay according to claim 55, wherein said cardiac specific isoform of troponin is cardiac specific troponin I, and said antibody specifically binds complexes comprising a reduced form of cardiac specific troponin I.

68. An assay according to claim 55, wherein said cardiac specific isoform of troponin is cardiac specific troponin I, and said antibody specifically binds complexes comprising

oxidized and reduced forms of cardiac specific troponin I.

69. An immunoassay for determining the presence or amount of complexes of a cardiac specific isoform of troponin in a patient sample, said immunoassay comprising:

a) contacting a sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds said cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C, and troponin T; or

contacting a sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds said cardiac specific isoform of troponin in a binary complex comprising one other troponin component selected from the group consisting of troponin I, troponin C, and troponin T; and, contacting the sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds said cardiac specific isoform of troponin in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C, and troponin T;

b) contacting the mixture of step (a) with a solid phase comprising at least one capture antibody which specifically binds said complexes which are bound to antibody conjugate or which specifically binds said complexes which are bound to antibody conjugate following binding to the capture antibody; and

c) generating a detectable signal from the antibody conjugate following binding to the solid phase of cardiac specific troponin complexes which are or become bound to antibody conjugate, wherein said detectable signal is at least a factor of two larger than a minimum signal resulting from said antibody conjugate(s) binding to an equal number of free troponin components and/or troponin complexes which do not comprise said cardiac specific isoform of troponin, and wherein the detectable signal is related to the presence or amount of said complexes in said sample.

70. An immunoassay according to claim 69, wherein the patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

*2 cont*

71. An immunoassay according to claim 69, wherein said detectable signal is at least a factor of five greater than said minimum signal.

72. An immunoassay for determining the presence or amount of complexes of any cardiac specific isoform of troponin in a patient sample, said immunoassay comprising:

a) contacting a sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds a cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; or

contacting a sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds a cardiac specific isoform of troponin in a binary complex comprising one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; and contacting the sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds said cardiac specific isoform of troponin in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T;

b) contacting the mixture of step (a) with a solid phase comprising at least one capture antibody which specifically binds said complexes which are bound to antibody conjugate or which specifically binds said complexes which are bound to antibody conjugate following binding to the capture antibody; and

c) generating a detectable signal from the antibody conjugate following binding to the solid phase of cardiac specific troponin complexes which are or become bound to antibody conjugate, wherein said detectable signal is at least a factor of two larger than a minimum signal resulting from said antibody conjugate(s) binding to an equal number of free troponin components and/or troponin complexes which do not comprise said cardiac specific isoform of troponin, and wherein the detectable signal is related to the presence or amount of said complexes in said sample.

73. An immunoassay according to claim 72, wherein the patient sample is selected

from the group consisting of a blood sample, a serum sample, and a plasma sample.

74. An immunoassay according to claim 72, wherein said detectable signal is approximately equal for equal amounts of all cardiac specific isoforms of troponin.

75. An immunoassay according to claim 72, wherein said detectable signal is within a factor of 0.2 for equal amounts of all cardiac specific isoforms of troponin.

76. An immunoassay according to claim 72, wherein said detectable signal is within a factor of 2 for equal amounts of all cardiac specific isoforms of troponin.

77. An immunoassay according to claim 72, wherein said detectable signal is at least a factor of five greater than said minimum signal.

78. An assay for determining the presence or amount of complexes of any cardiac specific isoform troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds a cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said complexes, wherein said signal is at least a factor of two larger than a minimum signal resulting from said antibody binding to an equal number of free troponin components and/or troponin complexes which do not comprise said cardiac specific isoform of troponin, and wherein said signal is related to the presence or amount of said complexes in said sample.

79. An assay according to claim 78, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

80. An assay according to claim 78, wherein said signal is approximately equal for equal amounts of all cardiac specific isoforms of troponin.

81. An assay according to claim 78, wherein said detectable signal is within a factor of 0.2 for equal amounts of all cardiac specific isoforms of troponin.

82. An assay according to claim 78, wherein said signal is within a factor of 2 for equal amounts of all cardiac specific isoforms of troponin.

83. An assay according to claim 78, wherein said detectable signal is at least a factor of five greater than said minimum signal.

84. An assay according to either one of claims 55 or 78, wherein said antibody comprises a plurality of antibodies selected from the group consisting of recombinant and monoclonal antibodies.

*Sub B1* 85. An assay for determining the presence or amount of a free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds said free cardiac specific isoform of troponin, and which specifically binds said cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoform of troponin, wherein said signal is at least a factor of two larger than a minimum signal resulting from said antibody binding to an equal number of free troponin components which are not said cardiac specific isoform of troponin and/or troponin complexes which do not comprise said cardiac specific isoform of troponin, and wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoform of troponin in said sample.

*Sub B1* 86. An assay according to claim 85, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

87. An assay according to claim 85, wherein said detectable signal is at least a factor of five greater than said minimum signal. *NM*

*Sub B2* 88. An assay for determining the presence or amount of a free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

*D1*  
*2 cont.*  
*Sub D2*  
*Sub C3*

performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin I and/or cardiac specific troponin T, and which binds to cardiac specific troponin I and/or cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoform of troponin, wherein said signal is at least a factor of two larger than a minimum signal resulting from said antibody binding to an equal number of free troponin components which are not said cardiac specific isoform of troponin and/or troponin complexes which do not comprise said cardiac specific isoform of troponin, and wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoform of troponin in said sample.

89. An assay according to claim 88, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

90. An assay according to claim 88, wherein said signal is at least a factor of five greater than said minimum signal.

91. An assay for determining the presence or amount of free and complexed cardiac specific troponin I in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin I, and which binds to cardiac specific troponin I in a complex comprising at least one other troponin component selected from the group consisting of troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific troponin I, wherein said signal is at least a factor of two larger than a minimum signal resulting from said antibody binding to an equal number of free troponin components which are not said cardiac specific troponin I and/or troponin complexes which do not comprise said cardiac specific troponin I, and wherein said detectable signal is related to the presence or amount of said free and complexed cardiac specific troponin I in said sample.

*Sub D3*  
92. An assay according to claim 91, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

*Sub D4*  
93. An assay according to claim 91, wherein said detectable signal is at least a factor of five greater than said minimum signal. *JKM*

*Sub D4*  
94. An assay for determining the presence or amount of free and complexed cardiac specific troponin T in a patient sample, said assay comprising:

*2 cont*  
performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin T, and which binds to cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I and troponin C; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific troponin T, wherein said signal is at least a factor of two larger than a minimum signal resulting from said antibody binding to an equal number of free troponin components which are not said cardiac specific troponin T and/or troponin complexes which do not comprise said cardiac specific troponin T, and wherein said signal is related to the presence or amount of said free and complexed cardiac specific troponin T in said sample.

95. An assay according to claim 94, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

*Sub D4*  
96. An assay according to claim 94, wherein said detectable signal is at least a factor of five greater than said minimum signal. *JKM*

97. An immunoassay for determining the presence or amount of complexes of any cardiac specific isoform of troponin in a patient sample, said immunoassay comprising:

a) contacting a sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds to troponin complexes comprising at least two troponin components selected from the group consisting of troponin I, troponin C and troponin T; or

contacting a sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds to troponin binary complexes comprising two troponin components selected from the group consisting of troponin I, troponin C and troponin T; and contacting the sample with an antibody conjugate comprising a signal development element and an antibody which specifically binds troponin ternary complexes comprising troponin I, troponin C and troponin T, and;

b) contacting the mixture of step (a) with a solid phase comprising at least one capture antibody which specifically binds said complexes comprising a cardiac specific isoform of troponin which are bound to antibody conjugate, or which specifically binds said complexes comprising said cardiac specific isoform of troponin which are bound to antibody conjugate after binding to capture antibody; and

c) generating a detectable signal from the antibody conjugate following binding to the solid phase of said complexes comprising said cardiac specific isoform of troponin which are or become bound to antibody conjugate, wherein the detectable signal is related to the presence or amount of said complexes comprising said cardiac specific isoform of troponin in said sample.

98. An assay according to claim 97, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

99. An assay according to claim 97, wherein said detectable signal is approximately equal for equal amounts of all cardiac specific isoforms of troponin.

100. An assay according to claim 97, wherein said detectable signal is within a factor of 0.2 for equal amounts of all cardiac specific isoforms of troponin.

101. An assay according to claim 97, wherein said detectable signal is within a factor of 2 for equal amounts of all cardiac specific isoforms of troponin.

*Sub B5* 102. An assay for determining the presence or amount of all free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds any free cardiac

specific isoform of troponin, and which specifically binds any cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoforms of troponin, wherein said signal is related to the presence or amount of all free and complexed cardiac specific isoform of troponin in said sample.

*P2 cont'*

103. An assay according to claim 102, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

104. An assay according to claim 102, wherein said signal is approximately equal for equal amounts of all cardiac specific isoforms of troponin.

105. An assay according to claim 102, wherein said signal is within a factor of 0.2 for equal amounts of all cardiac specific isoforms of troponin.

106. An assay according to claim 102, wherein said signal is within a factor of 2 for equal amounts of all cardiac specific isoforms of troponin.

107. An assay for determining the presence or amount of all complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds any cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said complexed cardiac specific isoforms of troponin, wherein said signal is related to the presence or amount of all complexed cardiac specific isoforms of troponin in said sample.

108. An assay according to claim 107, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

*2 cont.*

109. An assay according to claim 107, wherein said signal is approximately equal for equal amounts of all cardiac specific isoforms of troponin.

110. An assay according to claim 107, wherein said signal is within a factor of 0.2 f for equal amounts of all cardiac specific isoforms of troponin.

111. An assay according to claim 107, wherein said signal is within a factor of 2 for equal amounts of all cardiac specific isoforms of troponin.

112. An assay according to either one of claims 93 or 107, wherein said immunoassay provides a quantitative assay for any complexed cardiac specific isoform of troponin.

113. An assay according to either one of claims 102 or 107, wherein said antibody comprises a plurality of antibodies selected from the group consisting of recombinant and monoclonal antibodies.

*Subj Cls*

114. An assay for determining the presence or amount of a free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds said free cardiac specific isoform of troponin, and which specifically binds said cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoform of troponin, wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoform of troponin in said sample.

115. An assay according to claim 114, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

116. An assay according to claim 114, wherein said signal is approximately equal for equal amounts of said free cardiac specific isoform of troponin and said complexed cardiac specific isoform of troponin.

*D6*  
~~117. An assay according to claim 114, wherein said signal is within a factor of 0.2 for equal amounts of said free cardiac specific isoform of troponin and said complexed cardiac specific isoform of troponin.~~

*2 cont. Sub C7*  
~~118. An assay according to claim 114, wherein said detectable signal is within a factor of 2 for equal amounts of said free cardiac specific isoform of troponin and said complexed cardiac specific isoform of troponin.~~

*2 cont. Sub C7*  
~~119. An assay for determining the presence or amount of a free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:~~

*X*  
~~performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin I and/or cardiac specific troponin T, and which binds to cardiac specific troponin I and/or cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T; and~~

*X*  
~~detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoform of troponin, wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoform of troponin in said sample.~~

*Sub D7*  
~~120. An assay according to claim 119, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.~~

*X*  
~~121. An assay according to claim 119, wherein said signal is approximately equal for equal amounts of said free cardiac specific isoform of troponin and said complexed cardiac specific isoform of troponin.~~

*X*  
~~122. An assay according to claim 119, wherein said signal is within a factor of 0.2 for equal amounts of said free cardiac specific isoform of troponin and said complexed cardiac specific isoform of troponin.~~

*X*  
~~123. An assay according to claim 119, wherein said signal is within a factor of 2 for equal amounts of said free cardiac specific isoform of troponin and said complexed cardiac specific isoform of troponin.~~

124. An assay for determining the presence or amount of free and complexed cardiac specific troponin I in a patient sample, said assay comprising:

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